ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

COATING OF ACETYLSALICYLIC ACID USING ORGANIC BINDERS ON Ti-6Al-4V AND CoCrMo MEDICAL IMPLANT MATERIALS

MASTER OF SCIENCE

EMİNE DEMİRTÜRK

BOLU, NOVEMBER 2016

ABANT IZZET BAYSAL UNIVERSITY

THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

DEPARTMENT OF CHEMISTRY

COATING OF ACETYLSALICYLIC ACID USING ORGANIC BINDERS ON Ti-6Al-4V AND CoCrMo MEDICAL IMPLANT MATERIALS

MASTER OF SCIENCE

EMİNE DEMİRTÜRK

BOLU, NOVEMBER 2016

APPROVAL OF THE THESIS

Coating of Acetylsalicylic Acid Using Organic Binders on Ti-6Al-4V and CoCrMo Medical Implant Materials submitted by **Emine DEMİRTÜRK** in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Chemistry, Abant Izzet Baysal University** by,

Examining Committee Members	**Signature**
Supervisor Assist. Prof. Dr. Erhan Budak Abant İzzet Baysal University	.....
Co-Supervisor Prof. Dr. İbrahim Belenli Abant İzzet Baysal University	.....
Member Assist. Prof. Dr. Sevim Demirözü Şenol Abant İzzet Baysal University	.....
Member Assoc. Prof. Dr. Cevher Altuğ Abant İzzet Baysal University	.....
Member Prof. Dr. Ekrem Yanmaz İstanbul Gelişim University	.....

November 03, 2016

Prof. Dr. Duran KARAKAS .....

Director, **Graduate School of Natural and Applied Sciences**

This thesis is devoted to my family and Meltem Erva TOKDEMİR

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Emine DEMİRTÜRK

_____

ABSTRACT

COATING OF ACETYLSALICYLIC ACID USING ORGANIC BINDERS ON Ti-6Al-4V AND CoCrMo MEDICAL IMPLANT MATERIALS
MSC THESIS
EMİNE DEMİRTÜRK
ABANT IZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: ASSIST. PROF. DR. ERHAN BUDAK)
(CO-SUPERVISOR: PROF. DR. İBRAHİM BELENLİ)
BOLU, NOVEMBER 2016

Acetylsalicylic acid is used as analgesic. Besides, it has anti-bacterial activity. The aim of this work is to coat medical implants with acetylsalicylic acid to make use of these properties. These coatings were applied by obtaining adherence of the coating to the substrate via chemical and physical bonding. In the first part of the study, medical implant materials were coated physically by using dip coating method. In the second part of the study, coatings were made chemically by using organic binders. Ethyl-2-cyanoacrylate was used as a binder because it has bacteriostatic property. In this study, the surfaces of applied coatings were investigated by powder X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and visual inspection.

KEYWORDS:
Acetylsalicylic Acid, Implant, Coating, Ethyl-2-cyanoacrylate

ÖZET

ORGANİK BAĞLAYICI KULLANILARAK Ti-6Al-4V VE CoCrMo MEDİKAL İMPLANT MATERYALLERİN ASETİLSALİSİLİK ASİT İLE KAPLANMASI
YÜKSEK LİSANS TEZİ
EMİNE DEMİRTÜRK
ABANT İZZET BAYSAL ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ
KİMYA ANABİLİM DALI
(TEZ DANIŞMANI: YRD.DOÇ. DR ERHAN BUDAK)
(İKİNCİ DANIŞMAN: PROF. DR İBRAHİM BELENLİ)
BOLU, KASIM - 2016

Asetilsalisilik asit ağrı kesici olarak kullanılmasının yanı sıra anti bakteriyel özellik gösteren bir ilaçtır. Bu çalışmanın amacı, medikal implantları bu özelliklerinden dolayı aspirinle kaplamaktır. Böylece implant yüzeyinde bakterilerin biyofilm oluşturmasının önüne geçmek amaçlanmıştır. Kaplamalar fiziksel ve kimyasal olarak uygulandı. Bu çalışmanın ilk kısmında, medikal implant malzemeleri daldırarak kaplama yöntemi kullanılarak fiziksel olarak kaplandı. İkinci kısımda ise kaplamalar organik bağlayıcı kullanılarak kimyasal olarak yapıldı. Bakteriyostatik özellik gösterdiği için bağlayıcı olarak etil 2-siyanoakrilat kullanıldı. Bu çalışmada uygulanan kaplamalar X-ışını kırınımı (XRD), Fourier Dönüşümlü Kızılötesi Spektroskopisi (FTIR) ve Taramalı Elektron Mikroskopu kullanılarak incelendi.

ANAHTAR KELİMELER:
Asetilsalisilik Asit, İmplant, Kaplama, Etil-2-siyanoakrilat

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

LIST OF ABBREVIATIONS AND SYMBOLS

ASA : Acetylsalicylic acid or Aspirin

CH_3COOH : Acetic acid

E2CA : Ethyl-2-cyanoacrylate

FT-IR : Foruier-Transform Infrared Spectroscopy

HBO_3 : Boric Acid

HCL : Hydrochloric acid

HNO_3 : Nitric acid

H_2O_2 : Hydrogen peroxide

H_2SO_4 : Sulphuric acid

H_3PO_4 : Phosphoric acid

ICDD : The International Centre for Diffraction Data

PBS : Phosphate Buffered Saline

SEM : Scanning Electron Microscopy

XG : Xanthan Gum

XRD : X-Ray Diffractometer

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my supervisor Assist. Prof. Dr. Erhan BUDAK and co-supervisor Prof. Dr. İbrahim BELENLİ and Hüsamettin ÇAKICI for their guidance, advice, criticism, encouragements and insight throughout the research.

I would like to thanks to my friends for their useful helps.

I would also like to special thanks Osman Yerebasmaz and my parents, for their unconditional love, endurance and support throughout my education.

1. INTRODUCTION

1.1 Coating

A coating is a layer of certain thickness that is applied in order to cover the surface of a material. The aim of coating applications may be decorative, functional, or both. The coating may cover the substrate partly or completely depending on the requirement of the process.

Functional coatings may be applied for modification the surface properties of the substrate, such as adhesion, wettability, corrosion resistance, or wear resistance.

Major types of surface coating are;

- Conversion Coatings (oxidation, anodizing)
- Metal Coatings (electrochemical, electroless)
- Thermal Coatings (carburizing, flame spraying)
- Deposition (Physical Vapor Deposition, Chemical Vapor Deposition)
- Organic Coatings

1.2 Implant Coating

Orthopaedic implants and prostheses are highly favorable to infection (Matl et al., 2008). Infection of orthopaedic implants and prostheses is a medical problem that has concerned people since their first use over two-and-a-half millennia ago. Fixation and infection have been major problems with such medical implants right from the beginning of implant practices (Odekerken et al., 2013). Infection means that microorganisms have entered a wound and caused damage by destruction of healthy cells in the surrounding tissue (Gooch, 2010). In many cases, it may result in serious discomfort, limb amputation, illness and it may even result in death of patient in many cases (Odekerken et al., 2013).

Bacterial biofilms are complex microbial communities and they require a challenge for treatment (Bakri et al., 2008). The primary stage of bacterial biofilm generation is the housing of a planktonic bacterium on the surface of the implantable device. After adhering, the bacterium begins to divide and encapsulate itself for defense against the host organism's immune response. In the second stage, the present bacterium starts to form colonies increasing the internal pressure in the biofilm and starts to expand. Planktonic bacteria are released from the biofilm. The bacteria released from biofilm can cause further infection in the surrounding tissues (Odekerken et al., 2013).

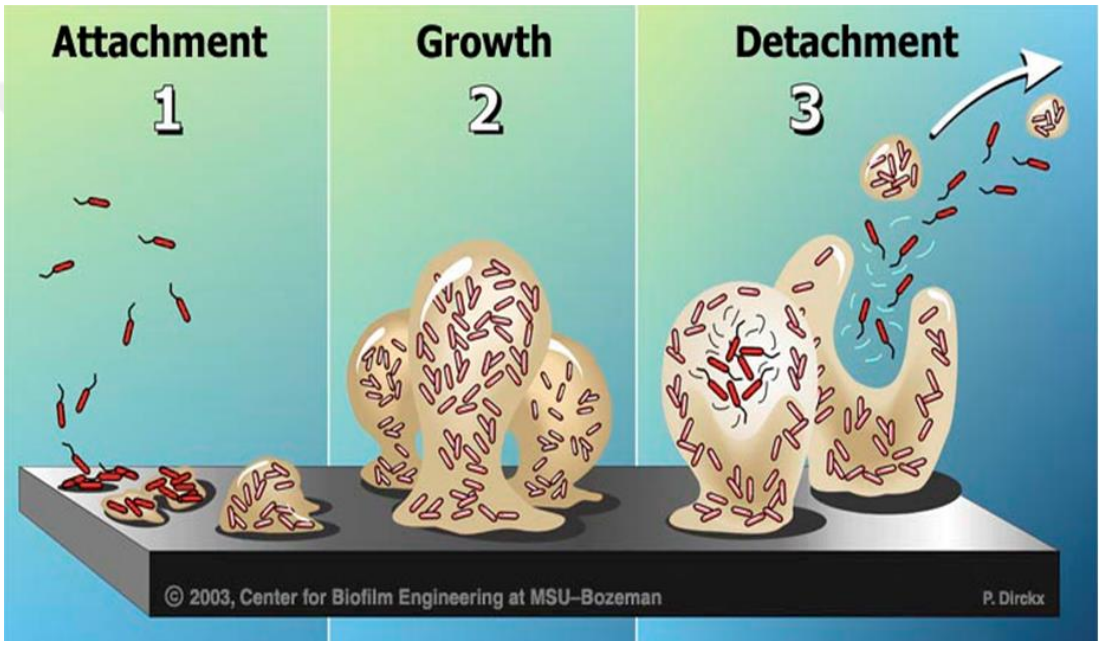


Figure 1.1. Schematic diagram of biofilm development (Stoodley and Dirckx, 2003)

Implants are coated to prevent the formation of a biofilm on implants' surface. Formation of biofilms on biomaterials offerings challenging complications in the field of medical implants (Matl, 2008). Biofilm formation can lead to delayed bone healing or ingrowth, nonunion of fractures, and implant loosening (Goodman et al., 2013). This can be prevented by antibiotics or antibacterial surface coatings.

1.3 The Types of Implants

In modern history, metals have been used as implants for more than 100 years. The use of metal plate for bone fracture fixation was first introduced by Lane in 1895 (Hermawan, 2011). In the field of orthopedics, the use of metal implants has significantly improved the quality of life for countless individuals. Critical factors for a successful implant application contain proper design, material selection, and biocompatibility (Campbell, 2003). Metallic biomaterials are exploited due to their inertness and structural functions; they do not possess bio-functionalities like blood compatibility and bioactivity. The orthopaedic implant metals are required to have excellent toughness, elasticity, rigidity, strength and resistance to fracture (Hermawan, 2011).

In the field of orthopaedic surgery, for restoring joint function, decreasing pain or stabilizing fractures metal implants are used. The most regularly used implants for these purposes are made of metallic alloys (Odekerken et al., 2013). The metallic materials such as stainless steels, Co–Cr alloys and titanium alloys are used as biomaterials in medicine (Akahori, 1998). Cobalt-chrome and titanium alloys are the most commonly used materials in total joint arthroplasty implants (Gallo et al., 2014). The stainless steel materials used for implants contain ~18wt% Cr and ~8wt% Ni making them stronger than the steel and more resistant to corrosion. Addition of molybdenum (Mo) has further improved its corrosion resistance. Titanium is featured by its light weight. Ti and its alloys, i.e. Ti-6Al-4V are known for their excellent tensile strength and surface corrosion resistance. Titanium alloyed with Ni, i.e. Nitinol, forms alloys having shape memory effect which makes them suitable in various applications such as dental restoration wiring (Hermawan, 2011). Metallic biomaterials have fundamentally three main employments. First one is artificial hip joints, screws, plates, second one is nails for internal fixation of bone fractures, and another one is dental implants (Akahori, 1998).

1.3.1 Ti-6Al-4V

Titanium (Ti) is presently employed in many implant parts in either pure or alloy form due to its strength, comparatively low stiffness, light weight, and relative

inertness (Campbell, 2003). Titanium has perfect mechanical properties, osteoconductivity, and biocompatibility (Park et al., 2014) (Katti, 2004). Titanium is a well-tolerated material compared to stainless steel and Co–Cr based alloys under in vivo conditions (Paital and Dahotre, 2009). Titanium dioxide (Ti_2O) phase which is extremely chemically stable forms on the surface of titanium and titanium alloys and due to this phenomenon, titanium and titanium alloys have the best biocompatibility among metallic biomaterials (Akahori, 1998).

1.3.2 CoCrMo

CoCrMo alloys are biocompatible materials. They are widely used as orthopedic implant materials in medicine such as hip joint and knee replacement by the reason of its superior mechanical properties, good wear and corrosion resistances. CoCrMo alloy has thin passive oxide film that spontaneously forms on the alloy surface and this situation is closely related to its excellent corrosion resistance (Türkan et al., 2006).

1.4 Adhesion

Adhesion is the attraction between two substances resulting from intermolecular forces that establish between them (Silva et al., 2011). The intermolecular forces that exist in adhesion is mainly van der Waals type forces. Mechanical, electrical, and diffusion phenomena may also occur at the adhesion level (Possart, 2005). The region between the adhesive and the adherend is referred as the interphase. The interphase has chemical and physical characteristics different from those of the bulk adhesive or adherend (Silva et al., 2011).

The adhesion of biological tissues is challenging in that the adhesive materials must satisfy several conditions. Adhesive materials must have minimal tissue toxicity, rapid adhesion property and must be biodegradable (Lim and Kim, 2015).

1.4.1 Cyanoacrylates

First cyanoacrylate was synthesized by German chemist Ardis in 1949. But it was used as tissue adhesive in 1959. Other forms (methyl-, ethyl-, isobutyl-, octyl-) were developed in the later years.

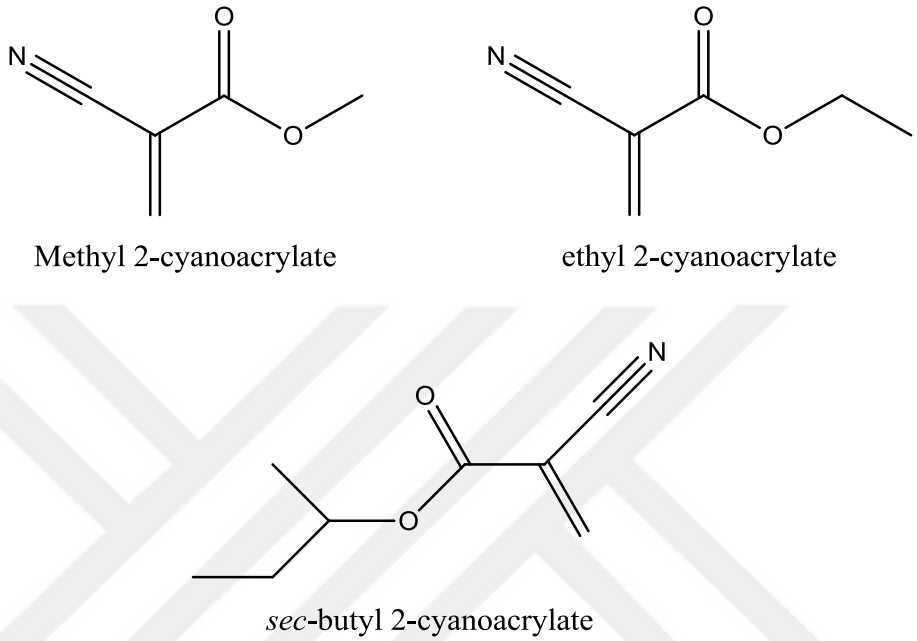


Figure 1.2. The structure of the cyanoacrylate tissue adhesives (Singer et al., 2008)

Cyanoacrylates are synthesized by condensation of cyano-acetate with formaldehyde in the presence of a catalyst, heat and vacuum (Davis et al., 2013) (Singer et al., 2008).

Cyanoacrylates are used as structural adhesives for metals, plastics, rubbers, and ceramics and they are also used as surgical adhesives and coatings in medicine due to their bacteriostatic and hemostatic properties and their high reactivity in moist conditions (Lim and Lee, 2014). The cyanoacrylates have also been shown to have antimicrobial properties (especially against Gram-positive organisms that are responsible for most wound infection) both in vitro and in animal models (Singer et al., 2008). Cyanoacrylate based adhesives have been used for the fixation of bone grafts for recent several years. Octyl-cyanoacrylate adhesives are not bioabsorbable. Butyl and octyl-cyanoacrylate have less adhesive strength than ethyl-cyanoacrylate.

Ethyl-cyanoacrylate has a smaller lateral chain than the other surgical adhesives; therefore it has great adhesive strength and a short healing time (Saska et al., 2008).

1.5 Acetylsalicylic Acid

Aspirin is the most widely used drug in the world (Vane and Botting, 2003).

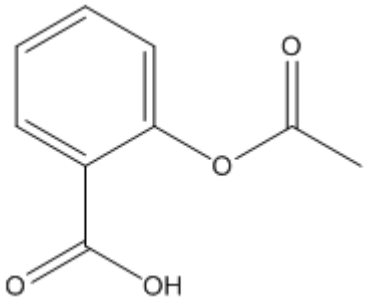


Figure 1.3. The skeletal representation of acetylsalicylic acid

Aspirin (acetylsalicylic acid), is as salicylate drug often used as analgesic to ease minor aches and pains, as an antipyretic to decrease fever, and as an anti-inflammatory medication (Rao et al., 2016). It has also hinders prostaglandrin synthetase (Lawal and Obaleye, 2007), (Mahdi et al., 2005). Paracetamol has analgesic and antipyretic action which is useful in the treatment of pain such as headache, toothache, rheumatism and neuralgia (Mahdi, 2010).

Table 1.1. Essential chemical properties of acetylsalicylic acid

Molecular Formula	$C_9H_8O_4$
Systematic IUPAC name	2-(acetyloxy) benzoic acid
Molar Mass	180.157 g/mol
Density	1,40 g/cm3
Melting point	135 °C (275 °F)
Boiling point	140 °C (284 °F) (decomposes)
Solubility in water	3 mg/mL (20 °C)

The historical account of willow tree (Salix sp.) goes back to early civilizations, particularly in Mesopotamia around 6000 years ago when plants were exploited for food and as a source of drugs (Mahdi et al., 2005). In the 18th century,

the modern era of aspirin discovery began. In 1828 at the University of Munich, Buchner obtained a yellowish substance, which he called salicin, the Latin name for the willow. Also, French pharmacist Henri Leroux obtained the pure crystalline form of salicin in 1829 (Mahdi, 2010).

Figure 1.4. 100-year-old pollarded willow trees (Jack, 1997)

Raffaele Piria resolved the chemical structure of salicin as a glucosidic salicyl alcohol. Then he oxidized salicyl alcohol into salicylic acid. Felix Hoffman, working at the Bayer company in Germany, made the acetylated form of salicylic acid in1897 (Szczeklik, 2006). This drug was named "Aspirin and became the most widely used medicine of all times. (Mahdi, 2010)

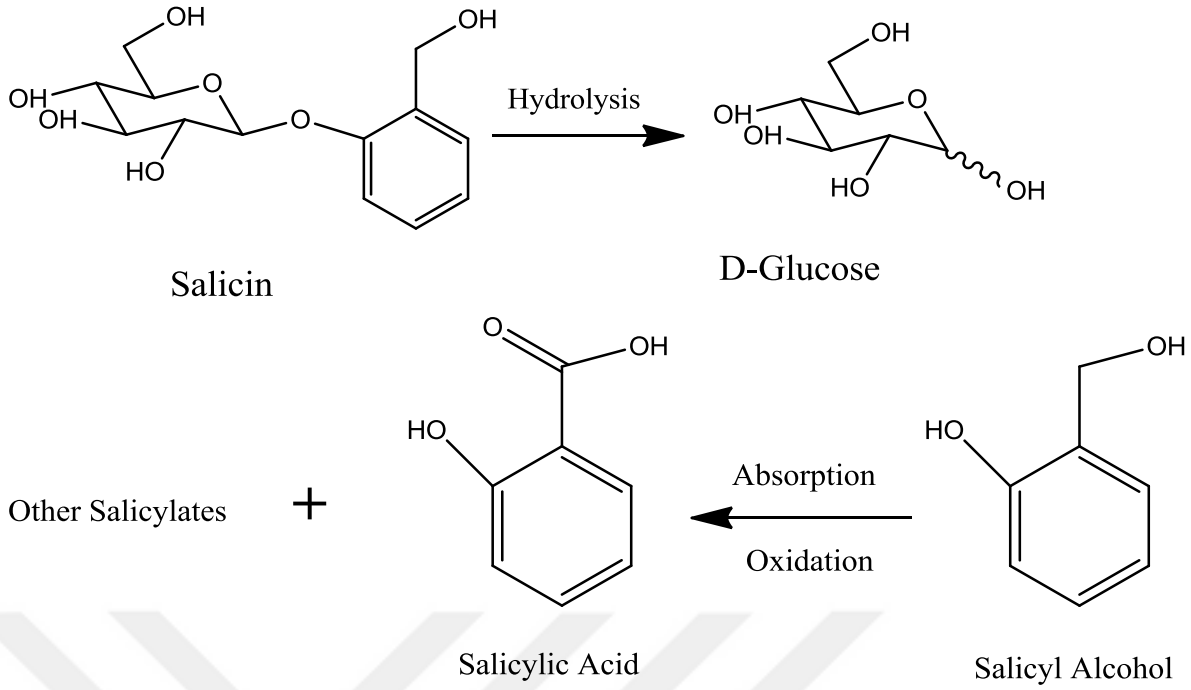


Figure 1.5. Hydrolysis of salicin

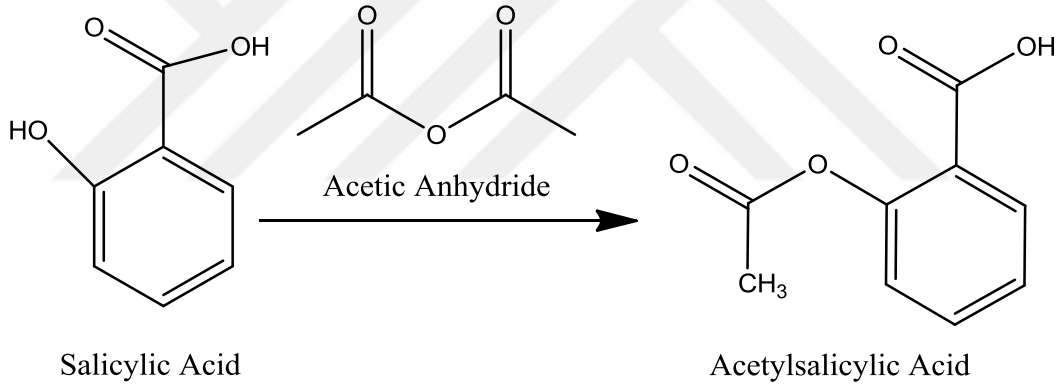


Figure 1.6. Acetylation of salicylic acid

The Bayer Company registered the product of Aspirin on 6 March 1899 and then in 1915 this product was available to the public without prescription (Mahdi et al., 2005).

2. AIM AND SCOPE OF THE STUDY

There are many ways to coat medical implants for different areas of usage. In this study we have proposed coating of implant materials with acetylsalicylic acid to prevent or decrease biofilm formation on medical implant surface. Surface of coatings were not smooth but durable. Bacterial growth was investigated with and without acetylsalicylic acid coating process. Finally, it was observed that coating with acetylsalicylic acid has a reducing effect on the biofilm formation.

3. EXPERIMENTAL

3.1 Chemicals

Acetylsalicylic acid (Bayer), Xanthan Gum (Sigma Aldrich), Ethyl-2-cyanoacrylate (Pattex), Ethanol (Merck), Boric acid (Sigma Aldrich), Acetic acid 100% (Sigma Aldrich), Nitric acid 65% (Merck), Phosphoric acid (Merck), Hydrogen peroxide 35% (Merck), Sulphuric acid 95-97% (Merck), Hydrochloric acid 36,5-38% (Sigma Aldrich).

3.2 Physical Coatings

3.2.1 Ethanol-water solution

10mL ethanol-water solution was prepared (9mL ethanol and 1mL pure water) and was added and dissolved in this solution. Ti-6Al-4V and CoCrMo metal alloy substrates in the form of disc were dipped into this solution then dried in open air.

3.2.2 Xanthan Gum

XG1- 5 mg ASA was ground and mixed with 5 mg XG. 2-3 mL water was added to this powder mixture. After mixing process, a gel-like solution was obtained. This solution was applied to on the implant surface as a thin film layer.

As a second way of coating with XG2- 5 mg XG and water were mixed and a gel-like solution was obtained. This solution was applied to on the implant surfaces for surface wetting. 3 mg ASA was ground and distributed uniformly on wetted surface and dried.

In other experimental work, XG3- 5 mg XG and 3 mg ground ASA were dissolved in water. This solution was dropped onto the implant surface and this plate was heated to 80^0C by hot plate until dried

XG4- 5 mg ASA was ground and 5 mg XG was mixed in a mortar. This powder mixture was dusted onto the surface of implant and this implant was placed in ultrasonic water vapor bath.

XG5- 3mg ASA was grinded and 5 mg XG was mixed in a mortar. This powder mixture was dusted on the surface of implant and this implant was placed in ultrasonic water vapor bath.

3.3 Chemical Coating

E2CA was tested for adhesion. A couple of drops of cyanoacrylate was dropped onto the surface of the implant disc and waited one hour in open air to dry.

Experiments from C1 to C5 were studied for determination of suitable ratio of ASA and E2CA.

C1- 25 mg ASA and 8 drops of E2CA were mixed. After that this sticky solution was spread on to the etched surface of implant material.

C2- 10 mg ASA and 10 drops of E2CA were mixed. After that this sticky solution was spread on to the etched metal surface of implant material.

C3- 10 mg ASA and 15 drops of E2CA were mixed. After that this sticky solution was spread on to the etched metal surface of implant material.

C5- 5 mg ASA and 12 drops of E2CA were mixed. One drop of mixture was spread to the metal surface of implant material. Same procedure at the same amounts was applied to C6 and C7 coatings to show repeatable accuracy. Three coated materials' weight and thickness values were measured and recorded.

C8- 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the metal surface of implant material and coated by spin coating at 4000 rpm spin speed.

C9 and C10- 1 mg ASA was dusted onto the metal surface of implant material, 1 drop of E2CA was dropped on to ASA and coated by spin coating at 4000 rpm spin speed.

In this part C13, C13/2 and C13/3 coating studies were performed to see the effect of multiple coating.

C13- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the metal surface of implant material and coated by spin coating at 4000 rpm spin speed.

C13/2- The material which is called C13 was coated second time by the same procedure.

C13/3- The material which is called C13/2 was coated third time by the same procedure.

C13, C14 and C15 coatings were performed for choosing suitable spin speed.

C14- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the metal surface of implant material and coated by spin coating at 2000 spin speed.

C15- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the metal surface of implant material and coated by spin coating at 1000 spin speed.

C16- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the surface of etched Ti-6Al-4V implant material and coated by spin coating at 4000 rpm spin speed.

C17- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the surface of etched CoCrMo implant material and coated by spin coating at 4000 rpm spin speed.

C19- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the surface of etched CoCrMo implant material and waited for drying.

C20- 3 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped on to the surface of etched Ti-6Al-4V implant material and waited for drying.

3.4 Examination of Drying Temperature

Different temperatures were tested to determine suitable drying temperature. 3 mg ASA and 5 drops of E2CA were used for each coating trial. 3 drops of mixture which contains ASA and E2CA was dropped to the surface of polished and etched discs with 2,15cm diameter. Drying processes were applied at 90^0C, 60^0Cand 40^0C temperatures in an oven for 20 minutes.

3.5 Bacteria Incubation Growth on the Coated Implant

Ti1 – Ti-6Al-4V plate was polished and etched with acidic solution.

Co1 – CoCrMo plate was polished and etched with acidic solution.

Ti2 - Ti-6Al-4V plate was polished and etched with acidic solution. 3 drops of E2CA were dropped onto coating surface. This plate was coated by spin coating device at 4000 rpm spin speed.

Co2 - CoCrMo plate was polished and etched with piranha solution. 3 drops of E2CA were dropped onto coating surface. This plate was coated by spin coating device at 4000 rpm spin speed.

Ti3 - Ti-6Al-4V plate was polished and etched with aqua regia solution. 3 drops of E2CA were dropped onto coating surface.

Co3 - CoCrMo plate was polished and etched with acidic solution. 3 drops of E2CA were dropped onto coating surface.

Ti4 - Ti-6Al-4V plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface. This plate was coated by spin coating device at 4000 rpm spin speed.

Co4 - CoCrMo plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface. This plate was coated by spin coating device at 4000 rpm spin speed.

Ti5 – Ti-6Al-4V plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped to coating surface. This plate was coated in spin coating device at 4000 rpm spin speed. The coated plate was dried in the drying oven at 90^0C degree for 20 minutes. The coating process including drying step was repeated 4 times on the same sample.

Co5 – CoCrMo plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface. This plate was coated by spin coating device at 4000 rpm spin speed. The coated plate was dried at the drying oven at 90^0C degree for 20 minutes. The coating process including drying step was repeated 4 times on the same surface.

Ti6 – Ti-6Al-4V plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface.

Co6 – CoCrMo plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface.

Ti7 – Ti-6Al-4V plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface. The coating process was repeated 4 times on the same surface.

Co7 – CoCrMo plate was polished and etched with acidic solution. 5 mg ASA and 5 drops of E2CA were mixed. 3 drops of mixture were dropped onto coating surface. The coating process was repeated 4 times on the same surface.

Also bacteria incubation was performed at Abant İzzet Baysal University Faculty of Medicine. The bacteria (Staphylococcus epidermidis) were incubated on each implant at 37°C for 24 h. disks were incubated with S. epidermidis then implant materials were gently washed three times in PBS and then fixed in 2.5% glutaraldehyde for 2 h at 48C. The surfaces were washed twice in PBS. The discs were postfixed with 0.1% OsO4 for 1 h and then dehydrated with increasing concentrations of ethanol.

3.6 Polishing

Ti-6Al-4V and CoCrMo plates of implant material were mounted with phenolic resin bakelite powder by using mounting machine. These plates were sequentially polished using SiC coated abrasive papers (grit sizes of 240-600-1200-2400-4000) by polishing device.

Figure 3.1. Mounting Device

Figure 3.2. Polishing Device

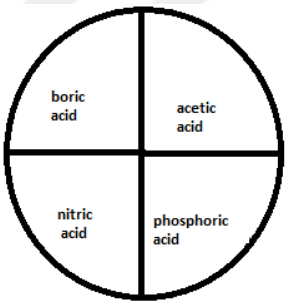
3.7 Etching

0,001 M H_3BO_3 (boric acid) solution was prepared. 0,06183 g boric acid was weighed, placed in a volumetric flask and added sufficient water to give a total volume of 100 mL.

0,1 M CH_3COOH (acetic acid) was prepared. 0, 57 mL concentrated acid (d: 1,05 g/mL) was transferred to a volumetric flask and water added to give a total volume of 100 mL.

0,01 M HNO_3 (nitric acid) solution was prepared. 0,07 mL concentrated acid (d: 1,39 g/mL and %65) was transferred to a volumetric flask and added water to give a total volume of 100 mL.

0,01 M H_3PO_4 (phosphoric acid) solution was prepared. 0,067 mL concentrated acid (d: 1,71 g/mL and %85) was transferred to a volumetric flask and added water to give a total volume of 100 mL.



A plate was divided to 4 equal parts with sticky tapes and 4 different acids were dropped onto each quarter of this plate. Acids did not etch surface of implant disc due to low acidity.

0, 5 M of HNO_3 solution was prepared. 3,47 mL concentrated acid (d: 1,39 g/mL and %65) was transferred to a volumetric flask and added water to give a total volume of 100 mL. Two drops of this solution were dropped on implant surface.

The stock solution of HNO_3 (14, 34 M) was dropped on the implant surface.

Piranha solution (3:1 H_2O_2- H_2SO_4) was used for Ti-6Al-4V plates. This solution was prepared as follows: 15 mL H_2O_2 (d: 1,13 g/mL %35) and 5 mL H_2SO_4

(d: 1,84 g/mL %97) were mixed and Ti-6Al-4V plates were put in this solution. Plates were etched ultrasonically 5 minutes.

Aqua regia solution (3:2 HCl-HNO_3) was used for CoCrMo plates. This solution was prepared as follows: 15mL HCl (d: 1,2 g/mL %36.5-38) and 10 mL HNO_3 (d: 1,3 g/mL %65) were mixed and CoCrMo plates were put in this solution. Plates were etched ultrasonically 5 minutes.

After etching process, plates were washed with distilled water, ultrasonically cleaned using distilled water and dried to maintain a uniform surface finish.

3.8 FT-IR Analysis

FTIR analyses were applied with "Infrared Fourier Transform Spectrometer" 1720 (Perkin-Elmer), the spectrometer was managed with a program "Spectrum v2.00" (Perkin-Elmer).

Figure 3.3. Perkin-Elmer Infrared Fourier Transform Spectrometer

3.9 XRD Analysis

XRD measurements were employed for the phase composition and crystal structure investigation. X-ray measurements were obtained by a Rigaku Multiflex + XRD diffractometer with monochromatic beam (wavelength of 1.54 Å) deriving a Cu Ka target. The scanning angle changes from 3^o to 50^o. It is speed and step increment is at 5^o/min, at 0.02^o in air atmosphere at the room temperature, respectively.

Figure 3.4. Rigaku MultiFlex 2kW X-Ray-Diffractometer

3.10 Scanning Electron Microscopy (SEM) Analysis

The surface morphology of ASA powders, etched metal surfaces and ASA coatings were studied by scanning electron microscope (SEM). SEM analyses were performed by using Electron Microscope JEOL JSM-6390LV (SEM). SEM operating voltage was 20 kV.

Figure 3.5. JEOL JSM-6390 LV Scanning Electron Microscope

SEM in a combination with an energy dispersive X-ray spectrometer (EDS) analysis was used for investigating the chemical composition of coatings and substrates.

3.11 Spin Coating

Some of the surface coatings were performed by spin coating device at various spin speeds in order to obtain thin uniform coatings. Spin coating operations were carried out by SCS Specialty Coating Systems Spincoat G3-8.

Figure 3.6. Spin coating device

4. RESULT AND DISCUSSION

4.1 Physical Coating

The physical coating methods were applied on the implant materials. Aspirin (ASA) was dissolved in ethanol-water solution and implant materials were dipped into this ASA loaded solution. After drying process, the implant materials were weight and amount of ASA determined (Table 4.1). It was observed that there was no change in amount of ASA. Although the solubility of ASA in ethanol-water solution is very high, this mixture is not suitable for coating of ASA because of lack of binding ASA to metal surface.

Table 4.1. The amount of ASA on implant material

Implant Type	Solvent	Mass of the implant disc before coating (g)	Mass of the implant disc after coating and drying (g)
Ti-6Al-4V	Ethanol-water	1.0215	1.0221
CoCrMo	Ethanol-water	1.9250	1.9253

Xanthan gum (XG) may be used as binder and carrier substance for dip coating process. XG makes rapid gelation when it exposed to water. In the first stage, interaction of XG/ASA and gelation were investigated. For achieving this aim, different amounts of XG and ASA were mixed using different methods. Among the XG coating techniques listed in the experimental chapter. Because of surface homogeneity XG5 was chosen as the most suitable method and XRD patterns was taken from XG5 coating.

Presence of ASA on the coated metal alloy implant disc was confirmed by XRD analysis by comparing with original XRD peaks of ASA (ICDD 12-0850) (Fig. 4.1). The main peaks of ASA (002, 012, 211, 202, $\bar{3}$02, 113, 022 and 312) were observed in XRD pattern of coated XG/ASA (Fig. 4.2) similar to literature (Aubrey-Medendorp et al., 2007). In addition, at 2θ= 46.540° XG peak was observed (Siddiqui and Tarannum, 2013). Although the coating appeared reasonable, the

binding between ASA/XG and implant disc is physically poor because of rough surface which decreased the interaction between XG and implant.

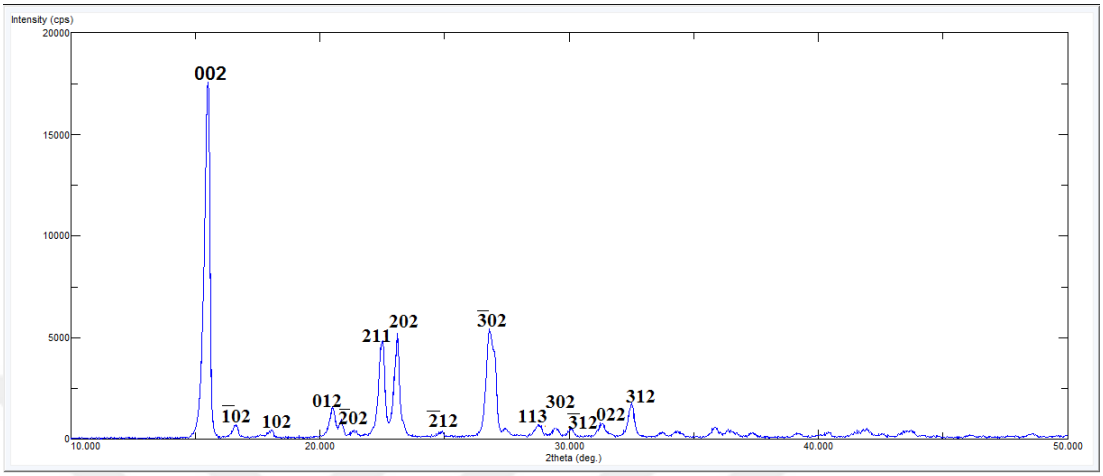


Figure 4.1. XRD pattern of ASA

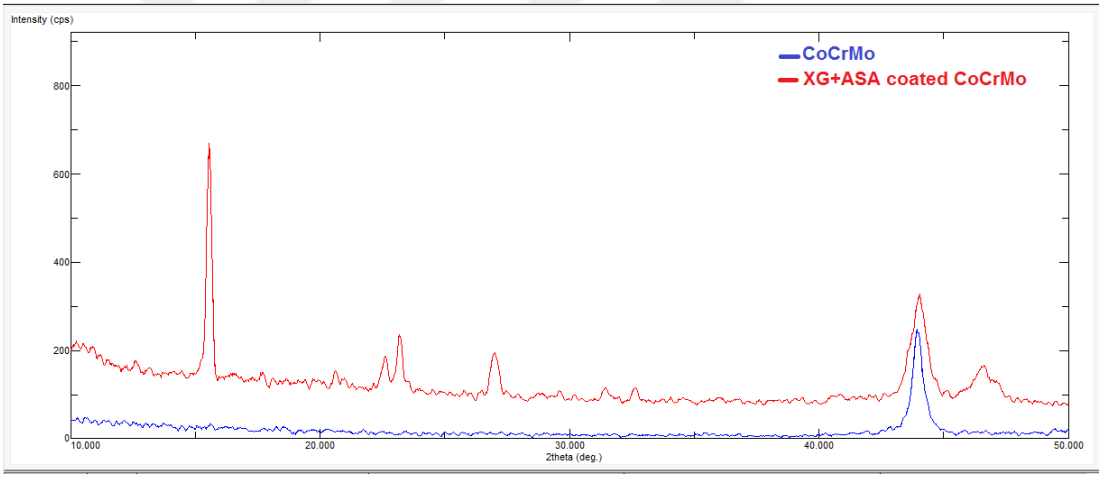


Figure 4.2. XRD patterns of CoCrMo and XG/ASA coated CoCrMo

Physical coating is not satisfactory, because the interaction between solvent (carrier substance-XG) and implant disc substrate is insufficient and adherence of coating onto the metal alloy substrate is poor.

4.2 Chemical Coating

Chemical methods were applied to increase interaction between implant surface and carrier substance. Ethyl-2-cyanoacrylate (E2CA) was used as chemical

binder. Cyanoacrylates yield better strengths when they bound to metals or other rigid surfaces. In addition, impact resistance becomes slightly better. FTIR spectroscopy was applied to characterize chemical interaction between implant and E2CA. The FTIR spectra of implant/E2CA and E2CA were compared individually (Fig 4.3 and Fig.4.4).

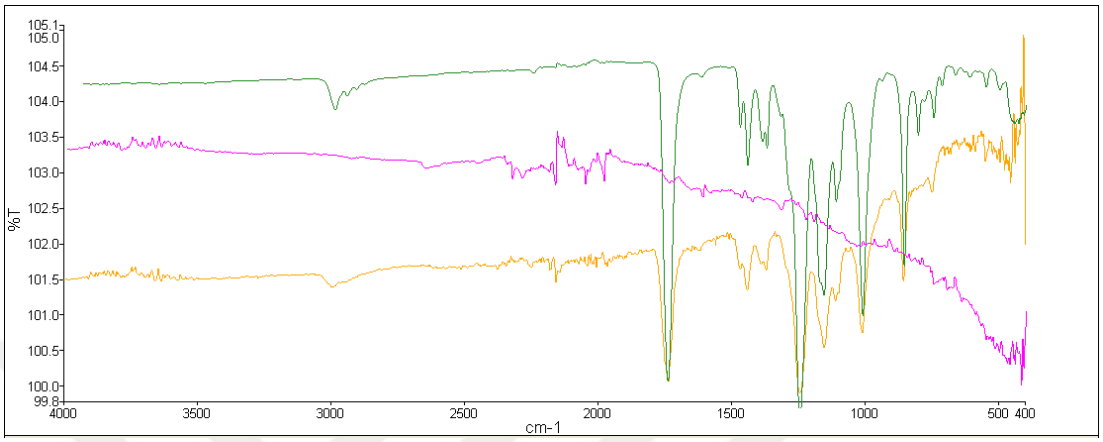


Figure 4.3. FT-IR spectrum of CoCrMo (pink line), E2CA (green line) and E2CA coated CoCrMo (orange line)

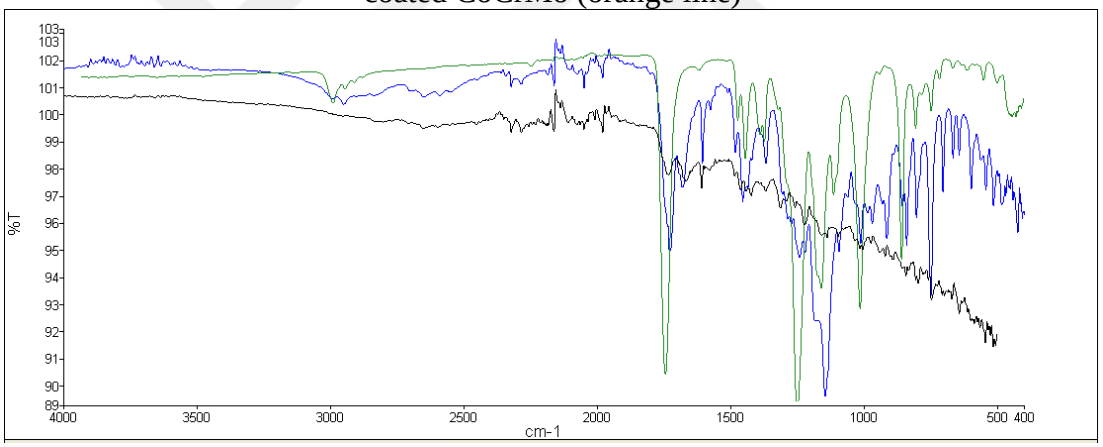


Figure 4.4. FT-IR spectrum of Ti-6Al-4V (black line), E2CA (green line) and E2CA coated Ti-6Al-4V (blue line)

As seen in these figures, the intensity of expected peaks is very low and E2CA peaks mask the expected peaks (Ti-O, Ti-H, Cr-O vibration modes), so the FTIR results were not distinctive. The used FTIR device is not enough to distinguish the expected peaks. However, XRD analyses were done to specify chemical interaction and crystallinity. The XRD diffractograms of implant/E2CA and implant were compared individually. In Ti-6Al-4V diffractogram (Fig. 4.5), the peaks at 2θ= 35.520, 38.640, 40.500° were observed and they were assigned to Ti-6Al-4V (Prabnu et al., 2014). Ti-6Al-4V/E2CA system was also examined by XRD and two different peaks were detected. Results can show that the peak at 2θ= 39.500 belongs to Ti_2O

phase (11-0218 ICDD card) and the peak at 44.280° belongs to TiH phase (65-0139 ICDD card).

In addition, CoCrMo and CoCrMo/E2CA structure were studied by XRD (Fig 4.6), too. Additional XRD peak at 2θ= 44.220° in XRD diffractogram can be labelled as Cr_3O 72-0528 ICDD card number. These results can show that there is a chemical interaction between implant material and E2CA.

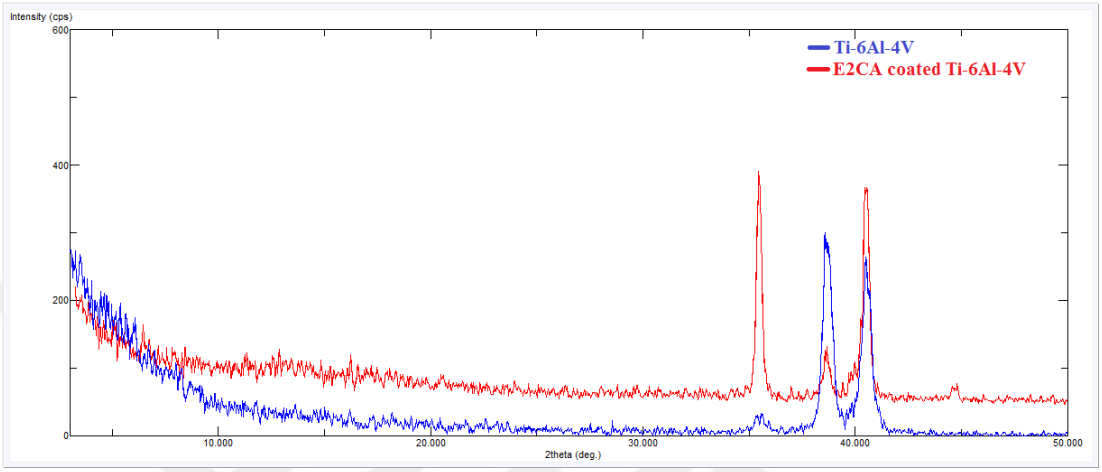


Figure 4.5. XRD patterns of Ti-6Al-4V and E2CA coated Ti-6Al-4V

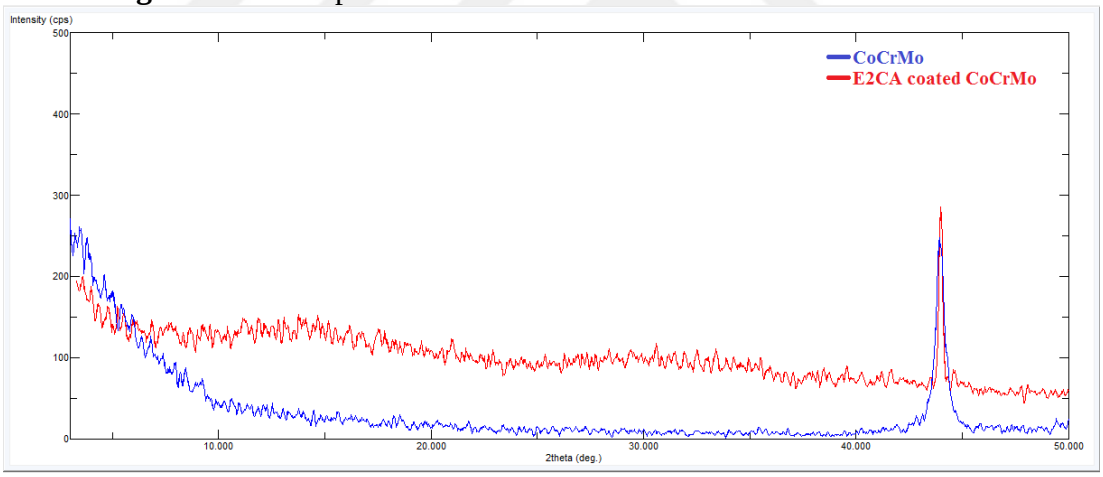


Figure 4.6. XRD patterns of CoCrMo and E2CA coated CoCrMo

Coating processes with E2CA and E2CA+ASA was performed by drop and spin coating methods.

4.2.1 Drop Coating

Different amounts of ASA and E2CA mixture were used for coating of CoCrMo implant. In XRD investigation (Fig. 4.7), 100, 002, 211, 202, 312 were assign to ASA and the peak at 2θ= 44.2° belonged to Cr_3O. With increasing E2CA

amount the Cr_3O peak intensity increased that this indicated that chemical interaction between E2CA and implant depends on E2CA amount.

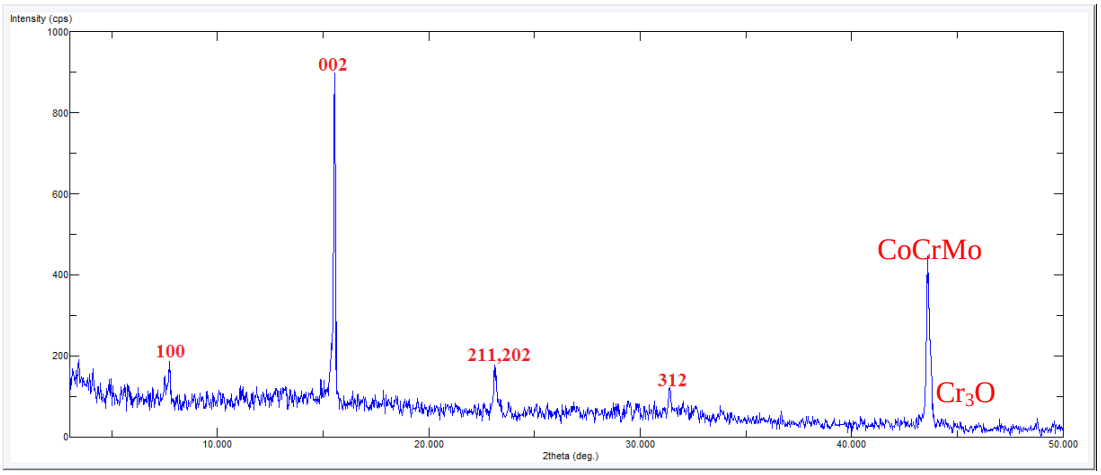


Figure 4.7. XRD pattern of coated CoCrMo by using drop method

ASA/E2CA coated Ti-6Al-4V by dropping method was also analyzed by XRD (Fig. 4.8). 002, 012, 211 and 202 peaks belonging to ASA could be indexed. In addition, at 2θ= 39.500 (Ti_2O) and 44.280° (TiH) were also seen.

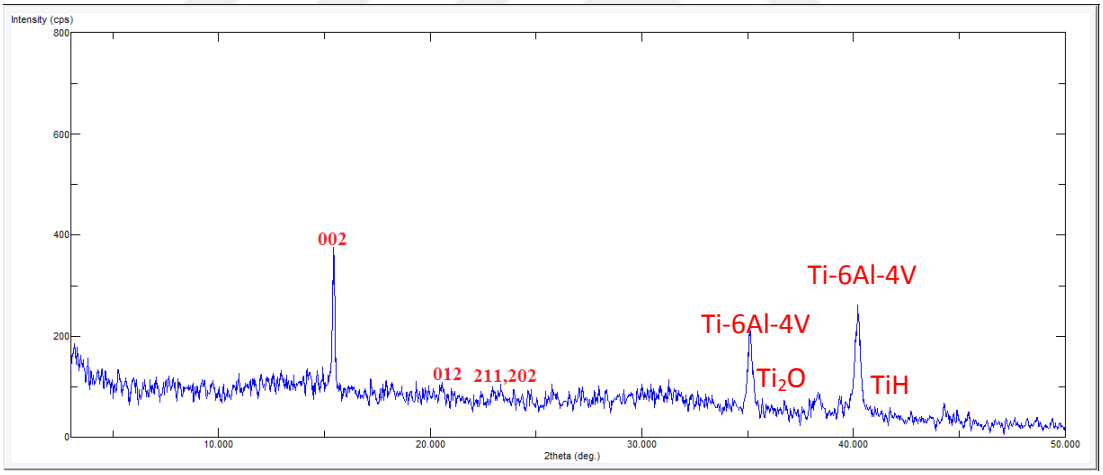


Figure 4.8. XRD pattern of coated Ti-6Al-4V by using drop method

Morphological examinations were done with SEM (Fig.4.9 a and b) and different ratio ASA/E2CA coatings images were compared. According to SEM images, the implant surface was completely coated in the dropping method but increasing amount of ASA caused to random crystal growth of ASA crystals (Fig. 4.9-b). For a thinner and more uniform coating, it may be necessary to modify ASA with a suitable surfactant chemical in order to prohibit excessive crystal growth.

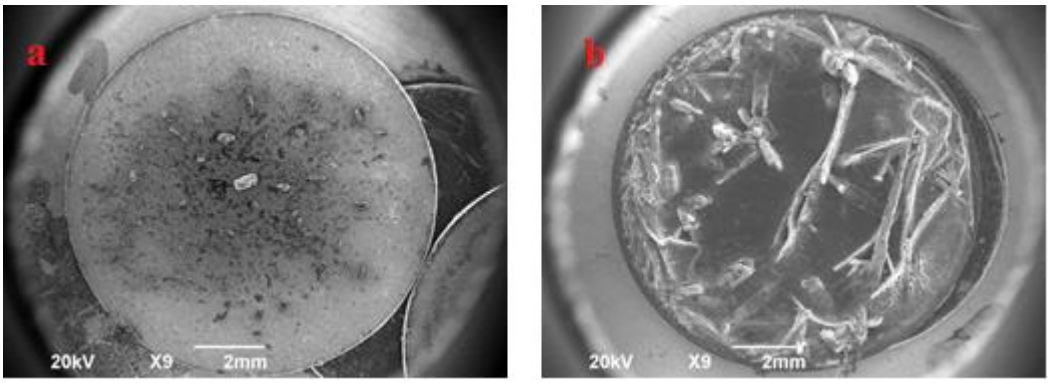


Figure 4.9. SEM images of coated implant discs by drop method. a) After dropping, excess of ASA/E2CA was discarded form surface by decantation and dried b) coated by dropping ASA/E2CA and dried without discarding the excess of ASA/E2CA.

4.2.2 Spin Coater

Spin coating is a common method to produce thin, uniform films on planar substrates (Hall et al., 1988). The ASA/E2CA films on implant discs were obtained by spin coating at 4000 rpm and determined by XRD (Fig. 4.10 and Fig. 4.11). The main peaks of ASA (100, 002, 211, 202,) and implants peaks were observed. In addition, the peaks at 2θ= 39.500 (Ti_2O) and 44.280° (TiH) were seen in figure 4.10, and in Figure 4.11 at 44.220° Cr_3O peaks detected due chemical interaction between E2CA and implant disc surface.

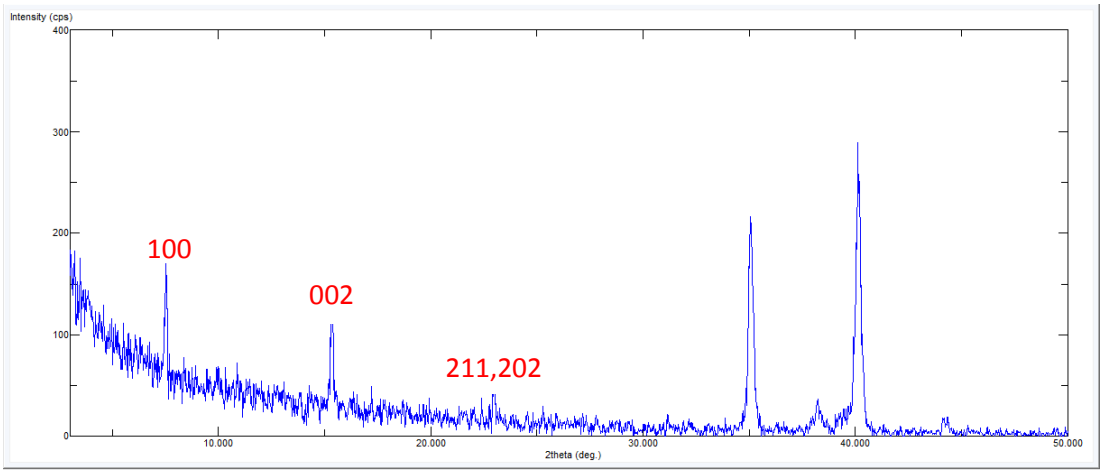


Figure 4.10. XRD pattern of ASA/E2CA coated Ti-6Al-4V implant by spin coater

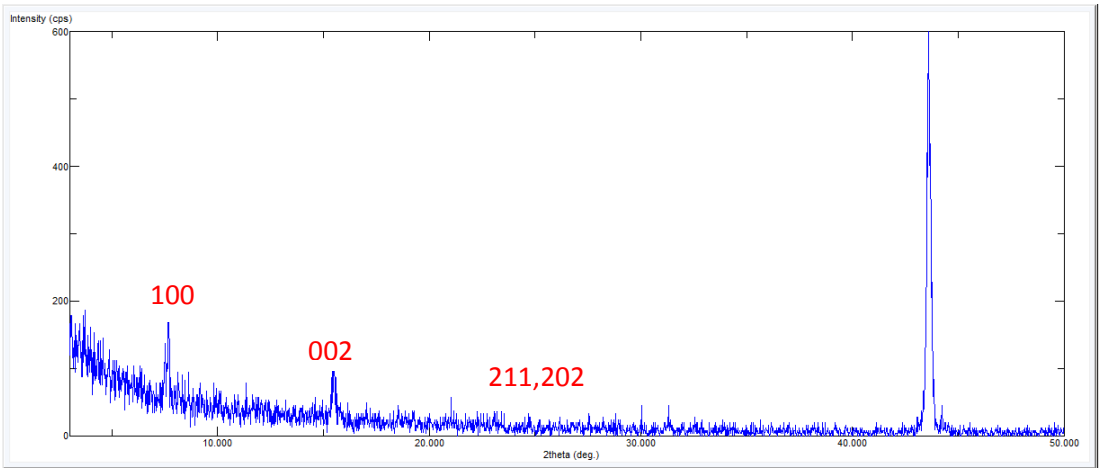


Figure 4.11. XRD pattern of ASA/E2CA coated CoCrMo implant by spin coater

SEM image (Fig. 4.12) illustrated that smoother surfaces can be obtained by using spin coater. In the spin coating process, the excess of coated material was thrown away from surface. Therefore, the amount of coating material remain on the coated surface is small, leading to thin coatings.

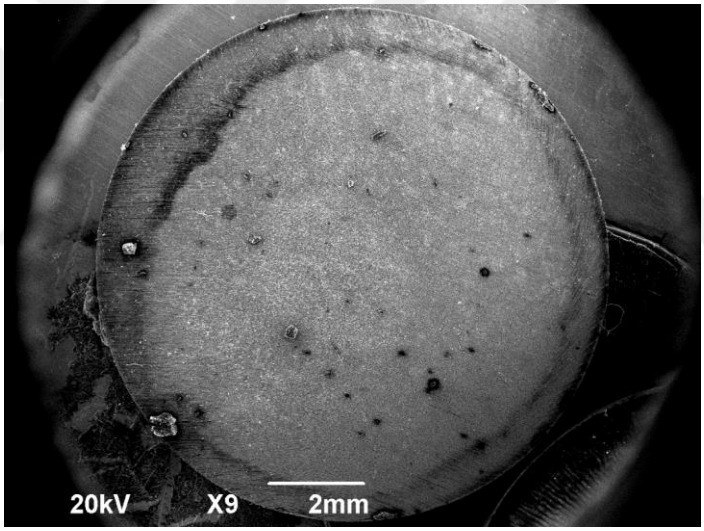


Figure 4.12. SEM image of coated implant which was coated by spin coater

4.3 Effect of Drying Temperature

In this part, the thermal stability of coated surface was examined at different temperature. Because main objective of this thesis is suitability of these implants to be used in vivo application the coated implants should be durable at human body temperature. The XRD analysis indicated that ASA coating was stable up to 60°C. Higher temperatures destroy the structure of ASA and E2CA. Due to low decomposition temperature of E2CA (54-56^{0}C) (Silva et al., 2011) ASA was affected

and decomposed, too. So that, the intensities of ASA peaks in XRD patterns were decreased at high temperatures (Fig. 4.13).

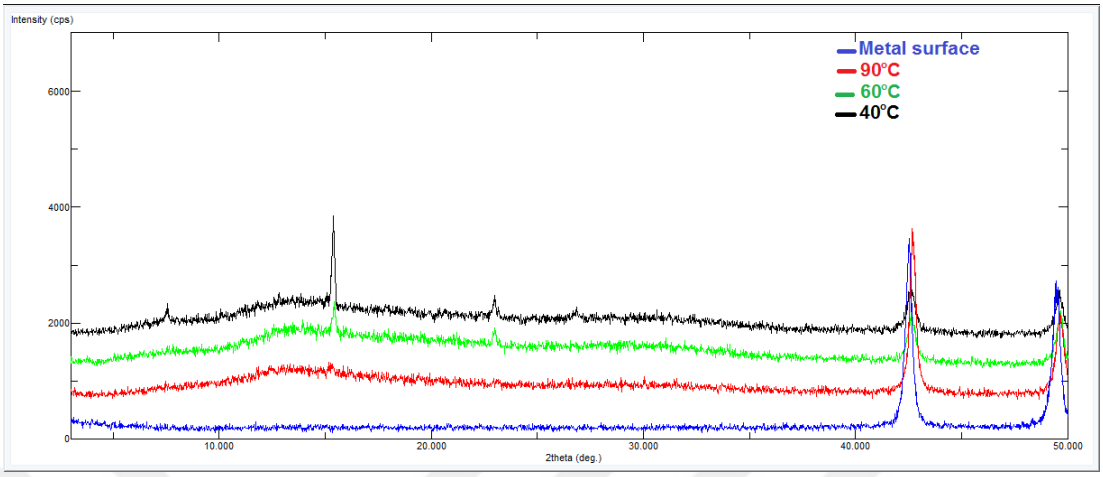


Figure 4.13. XRD pattern of ASA/E2CA coated metal which was applied different temperatures

4.4 Bacteria Incubation Growth on the Coated Implant

The bacteria (*Staphylococcus epidermidis*) were incubated on implant at 37°C for 24 h. The morphology of *S. epidermidis* on titanium alloy disks was examined using SEM.

4.4.1 Ti-6Al-4V Implant

SEM was used to evaluate existence *S. epidermidis* on the surface of both the bare (Fig 4.14) and the ASA/E2CA coated (Fig. 4.15) Ti-6Al-4V implant surfaces. Under different magnification, *S. epidermidis* was detected on the both Ti-6Al-4V and ASA/E2CA coated Ti-6Al-4V surface. The amounts of bacteria colony on the ASA/E2CA coated implant were significantly lower and showed a major decrease in the number of colonies.

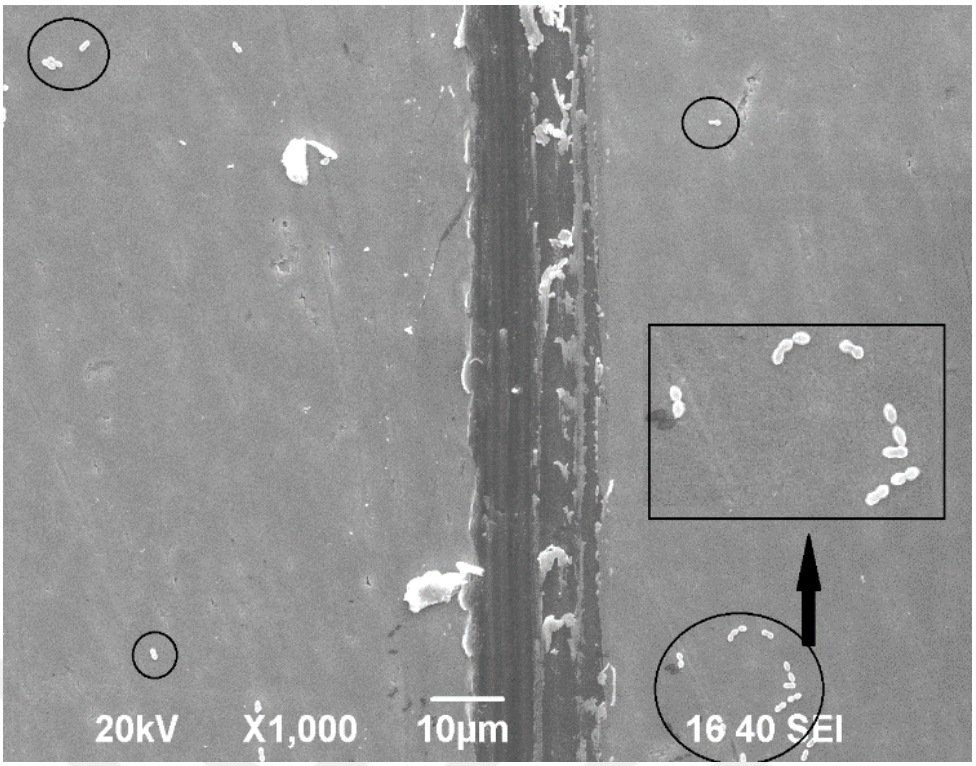


Figure 4.14. SEM image of Ti-6Al-4V which was incubated bacteria

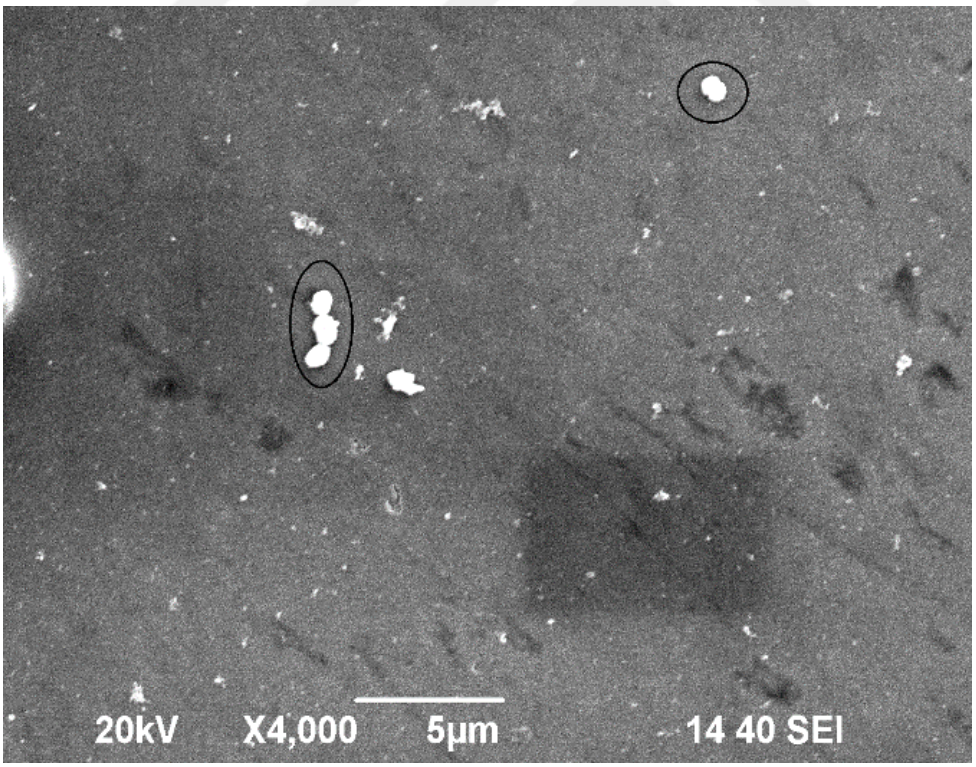


Figure 4.15. SEM image of ASA/E2CA coated Ti-6Al-4V which was incubated bacteria

4.4.2 CoCrMo Implant

S. epidermidis on CoCrMo alloy discs was examined using SEM and it was detected on CoCrMo alloy implant using different magnification. The colony number of *S. epidermidis* on the ASA/E2CA coated CoCrMo implant surface (Fig. 4.16) was smaller than bare (uncoated) CoCrMo implant surface (Fig. 4.17). One of the possible reasons for decreasing in colony could be the chemical interaction between ASA/E2CA and bacteria.

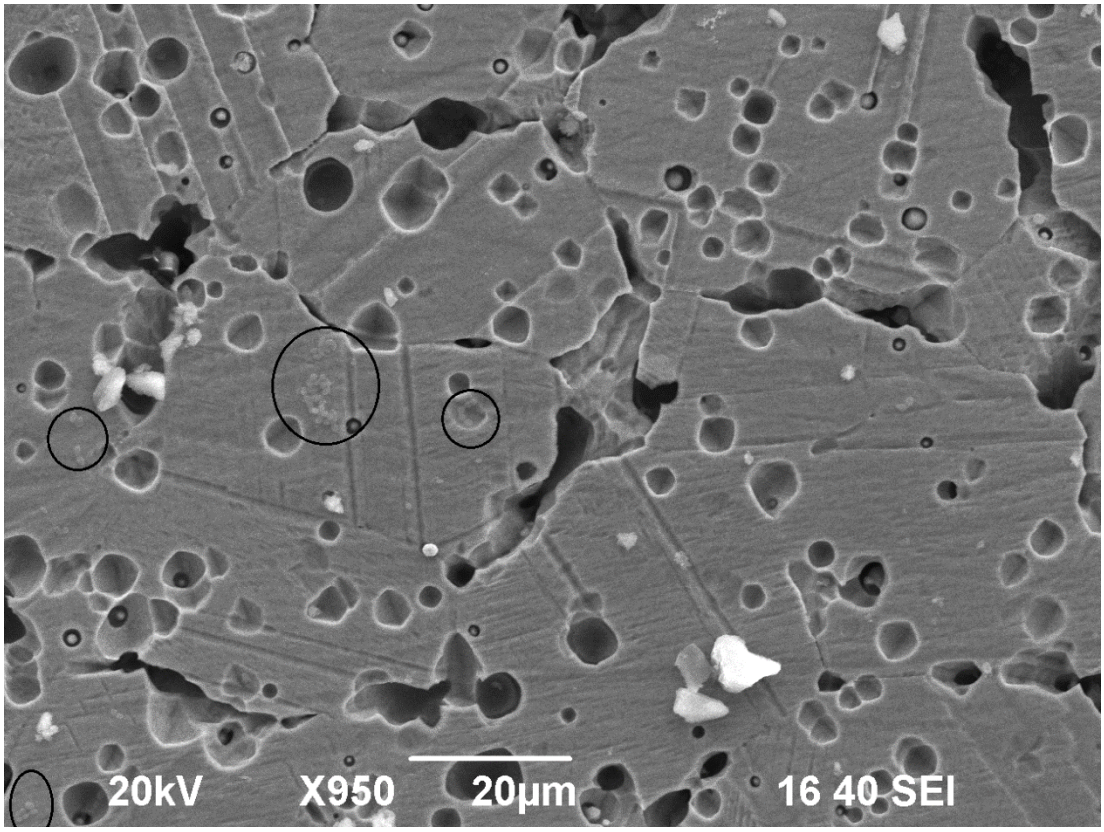


Figure 4.16. SEM image of ASA/E2CA coated CoCrMo which was bacteria incubated

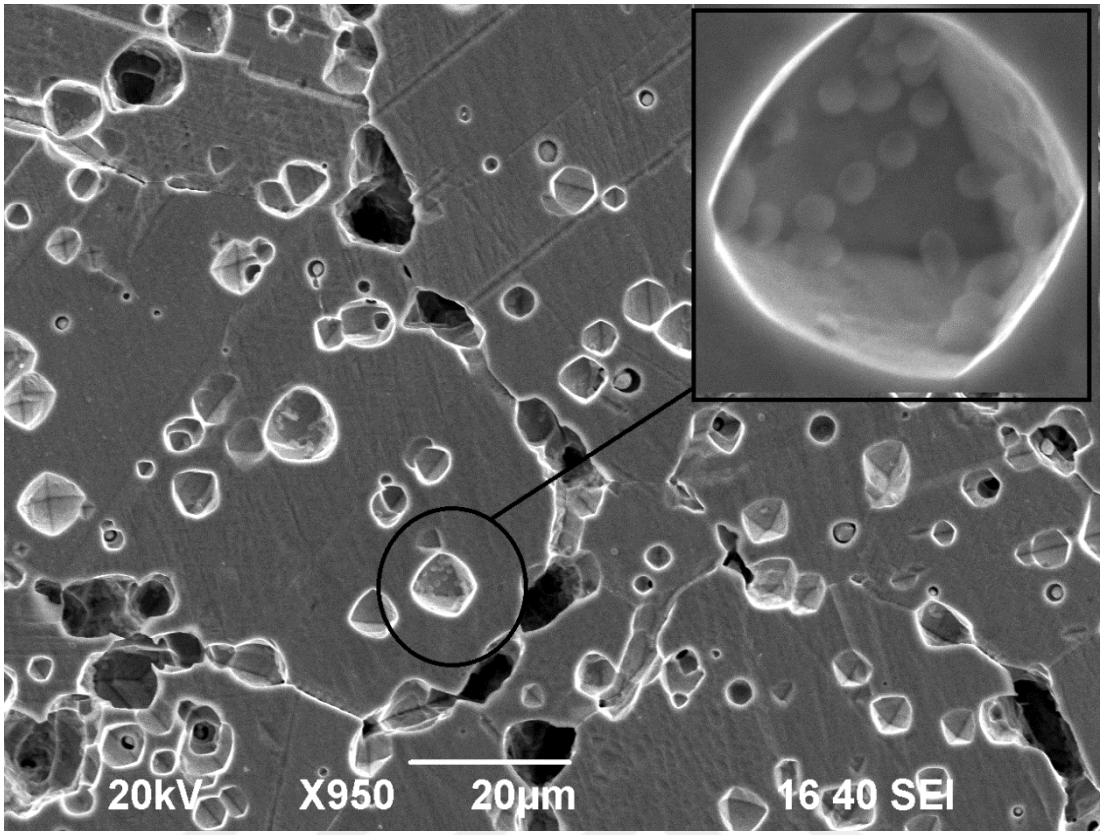


Figure 4.17. SEM image of uncoated CoCrMo which was incubated bacteria

5. CONCLUSION

In this study, two methods of coating (dip and spin coating) on Ti-6Al-4V and CoCrMo implants were experimentally investigated. Coatings were applied by using binder because ASA does not have binding property. XG and E2CA can be used as binder for coating of implants with ASA. E2CA has an extra advantage since it exhibits bacteriostatic property. E2CA give better results than XG because bonding of E2CA and ASA is occur chemically and this is proved by XRD. Even though the durability of coatings was good, the smoothness of surfaces was poor due to uncontrolled crystal growth of ASA. After coating process bacterial growth was investigated by using SEM. Examination of bacterial growth shows that coating with ASA causes a decreased bacterial growth and may probably have suppressing effect on biofilm formation on implant surfaces.

6. REFERENCES

Akahori T, Niinomi M (1998) “Fracture characteristics of fatigued Ti–6Al–4V ELI as an implant material”, Materials Science and Engineering: A, 243(1):237-243

Al-Bakri AG, Othman G, Bustanji Y (2009) “The assessment of the antibacterial and antifungal activities of aspirin, EDTA and aspirin–EDTA combination and their effectiveness as antibiofilm agents”, Journal of applied microbiology, 107(1):280-286

Aubrey-Medendorp C, Parkin S, Li T (2008) “The confusion of indexing aspirin crystals”, Journal of pharmaceutical sciences, 97(4):1361-1367.

Campbell AA (2003) “Bioceramics for implant coatings”, Materials today, 6(11):26-30

Davis KP, Derlet RW (2013) “Cyanoacrylate glues for wilderness and remote travel medical care”, Wilderness & environmental medicine, 24(1):67-74

Gallo J, Holinka M, Moucha CS (2014) “Antibacterial surface treatment for orthopaedic implants”, International journal of molecular sciences, 15(8):13849-13880

Gooch JW (2010) Barrier Dressings for Wounds In Biocompatible Polymeric Materials and Tourniquets for Wounds Springer New York

Goodman SB, Yao Z, Keeney M, Yang F (2013) “The future of biologic coatings for orthopaedic implants”, Biomaterials, 34(13):3174-3183

Hall DB, Underhill P, Torkelson JM (1998) “Spin coating of thin and ultrathin polymer films”, Polymer Engineering & Science, 38(12):2039-2045

Hermawan H, Ramdan D, Djuansjah JR (2011) “Metals for biomedical applications”, INTECH Open Access Publisher, (pp. 411-430)

Jack DB (1997) “One hundred years of aspirin”, The Lancet, *350*(9075), 437-439.

Katti KS (2004) “Biomaterials in total joint replacement”, Colloids and Surfaces B: Biointerfaces, 39(3):133-142

Lawal A, Obaleye JA (2007) “Synthesis, characterization and antibacterial activity of aspirin and paracetamolmetal complexes”, Biokemistri 19(1).

Lim JI, Kim JH (2015) “Enhanced biocompatibility and adhesive properties of modified allyl 2-cyanoacrylate-based elastic bio-glues”, Colloids and Surfaces B: Biointerfaces, 133:19-23

Lim JI, Lee WK (2014) “Enhanced biocompatibility and adhesive properties by aromatic amino acid-modified allyl 2-cyanoacrylate-based bio-glue”, Colloids and Surfaces B: Biointerfaces, 122: 669-673

Mahdi JG (2010) “Medicinal potential of willow: A chemical perspective of aspirin discovery”, Journal of Saudi Chemical Society, 14(3):317-322

Mahdi JG, Mahdi AJ, Bowen ID (2006) "The historical analysis of aspirin discovery, its relation to the willow tree and antiproliferative and anticancer potential", Cell proliferation 39.2:147-155

Matl FD, Obermeier A, Repmann S, Friess W, Stemberger A, Kuehn KD (2008) “New anti-infective coatings of medical implants”, Antimicrobial agents and chemotherapy, 6:1957-1963

Odekerken JC, Welting TJ, Arts JJ, Walenkamp GHIM, Emans PJ (2013) Modern orthopaedic implant coatings—their pro’s, con’s and evaluation methods ,Modern surface engineering treatments, InTech, New York

Paital SR, Dahotre NB (2009) “Calcium phosphate coatings for bio-implant applications: Materials, performance factors, and methodologies”, Materials Science and Engineering 66(1):1-70

Park JE, Park I S, Neupane MP, Bae TS, Lee MH (2014) “Effects of a carbon nanotube-collagen coating on a titanium surface on osteoblast growth”, Applied Surface Science, 292:828-836.

Possart W (2005) Adhesion: Current Research and Application, Wiley-VCH, Weinheim

Rao AS, Rao AP, Rao MK, Dadhich AS (2016) “Development and Validation of RP-HPLC Method for Assay of Acetylsalicylic Acid in Formulations”, European Journal of Biomedical and Pharmaceutical Sciences, 5:348-353

Saska S, Hochuli-Vieira E, Minarelli-Gaspar AM, Gabrielli MFR., Capela MV, Gabrielli MAC (2009) “Fixation of autogenous bone grafts with ethyl-cyanoacrylate glue or titanium screws in the calvaria of rabbits”, International journal of oral and maxillofacial surgery, 38(2):180-186

Siddiqui ZN, Tarannum S (2013) “Xanthan sulfuric acid: An efficient and biodegradable solid acid catalyst for the synthesis of bis (indolyl) methanes under solvent-free conditions”, Comptes Rendus Chimie, 16(9):829-837

Silva LF, Öchsner A, Adams RD (2011) “Handbook of adhesion technology”, Springer Science & Business Media

Singer AJ, Quinn JV, Hollander JE (2008) “The cyanoacrylate topical skin adhesives”, The American journal of emergency medicine, 26(4):490-496.

Stoodley P, Dirckx P (2003) Biofilm formation in 3 steps. http://www.biofilm.montana.edu/resources/images/multicellularextracellular/biofilm-formation-3-steps.html, (26.08.2016)

Szczeklik A (2006) The History of Aspirin: The Discoveries That Changed Contemporary Medicine, Pontifical Academy of Sciences, Vatican City

Türkan U, Öztürk O, Eroğlu AE (2006) “Metal ion release from TiN coated CoCrMo orthopedic implant material”, Surface and Coatings Technology, 200(16):5020-5027

Vane JR, Botting RM (2003) “The mechanism of action of aspirin”, Thrombosis research, 110(5):255-258.

7. APPENDICES

12-0850 Wavelength= 1.5405 *

C9H8O4

Acetylsalicylic acid

Rad.: CuKa1 λ: 1.5405 Filter: d-sp: Debye-S. 114.6

Cut off: 50.0 Int.: Film I/Icor.:

Ref: de Wolff, P., Technisch Physische Dienst, Delft, The Netherlands, ICDD Grant-in-Aid

Sys.: Monoclinic S.G.: $P2_1/c$ (14)

a: 11.43 b: 6.592 c: 11.41 A: 1.7339 C: 1.7309

α: β: 95.65 γ: Z: 4 mp:

Ref: Ibid.

Dx: 1.399 Dm: SS/FOM: F_{30} = 37 (.0120 , 68)

CAS #: 50-78-2. Also called: aspirin.PSC: mP84. Mwt: 180.16. Volume[CD]: 855.53.

d(A)	Int	h	k	l	d(A)	Int	h	k	l
11.40	35	1	0	0	2.734	2	2	2	1
5.68	100	0	0	2	2.648	2	4	0	2
5.29	6	1	0	2	2.600	4	2	2	2
5.20	2	1	1	1	2.497	4	4	1	1
5.00	2	1	1	1	2.488	4	3	2	0
4.89	2	1	0	2	2.458	6	4	1	2
4.30	20	0	1	2	2.398	2	3	2	1
4.23	4	2	0	2	2.294	2	4	1	2
4.13	4	2	1	1	2.246	2	4	1	3
3.93	35	2	1	1	2.226	4	3	2	2
3.83	8	2	0	2	2.157	6	0	3	1
3.79	2	3	0	0	2.145	4	4	2	1
3.56	4	2	1	2	2.127	2	1	3	1
3.31	20	3	0	2	2.116	2	4	0	4
3.29	25	3	1	0	2.078	2	5	1	1
3.23	4	3	1	1	2.049	2	0	3	2
3.17	2	1	2	0	2.030	2	2	3	1
3.08	2	1	1	3	1.965	2	4	2	2
3.02	2	3	0	2	1.948	2	3	1	5
2.957	4	3	1	2	1.900	2	0	3	3
2.848	6	0	2	2	1.869	4	1	2	5
2.820	2	1	0	4	1.837	<1	1	0	6
2.744	6	3	1	2	1.798	<1	3	1	5

Figure 7.1 ICDD Data Card of ASA

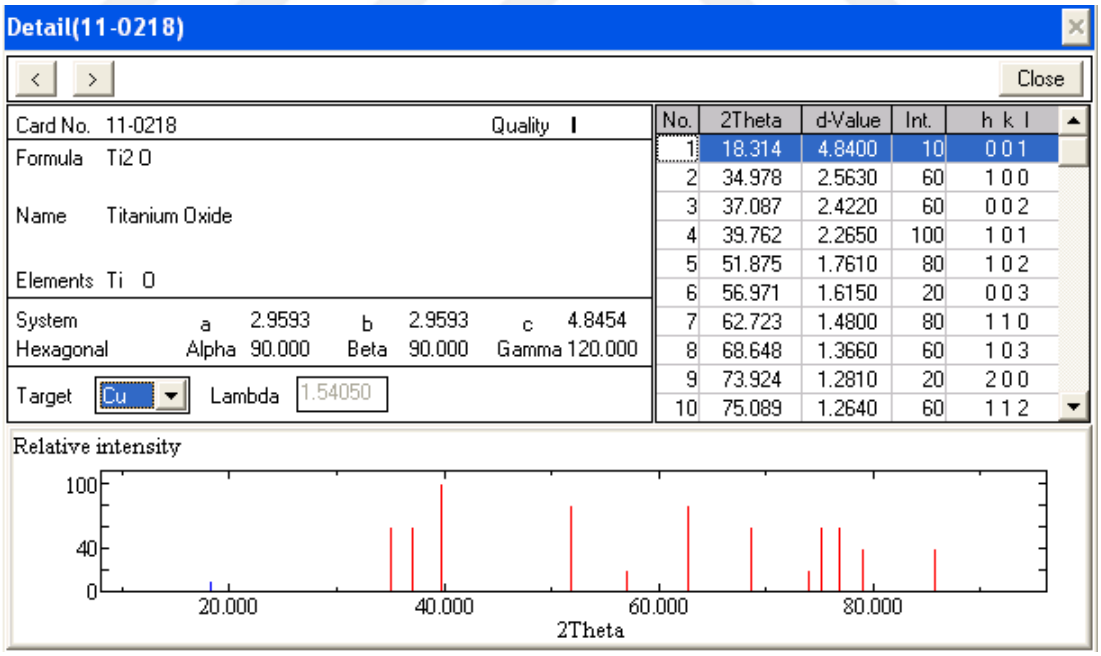


Figure 7.2 ICDD Data Card of Ti_2O

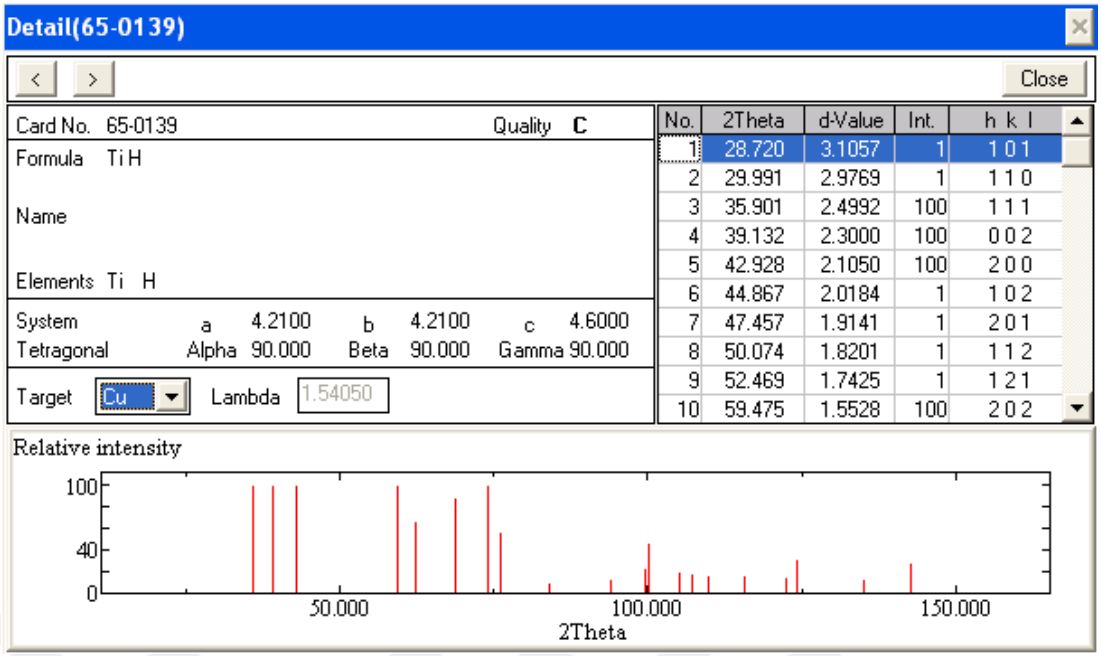


Figure 7.3 ICDD Data Card of TiH

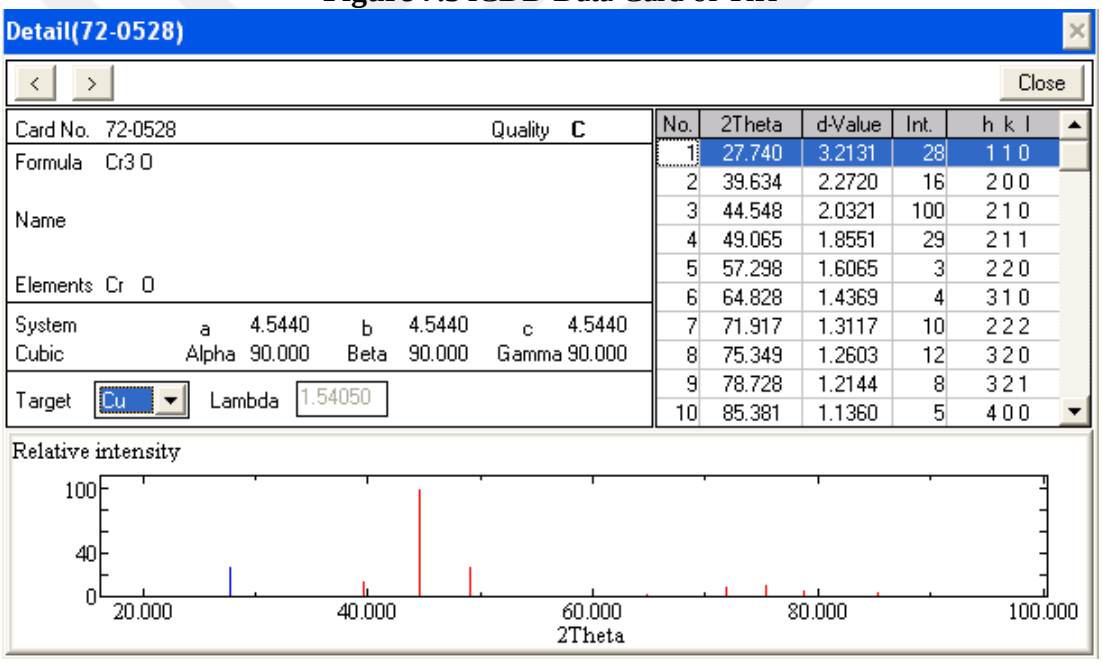


Figure 7.4 ICDD Data Card of Cr_3O

8. CURRICULUM VITAE

Name SURNAME **: Emine DEMİRTÜRK**

Place and Date of Birth **: Zonguldak / 01.01.1991**

- Bachelor's Degree **: Abant Izzet Baysal University 2013**

e-mail **: eminedemirturk@gmail.com**

Address **:Karamanlı Mah. Aydın Sk. Merkez-BOLU**